

Genetic diversity of Atlantic salmon (*Salmo salar*)
and European whitefish (*Coregonus lavaretus*)
in the Baltic Sea

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Academic dissertation

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Abstract

We described the patterns and extent of microsatellite DNA variation in historical and present-day Atlantic salmon (*Salmo salar* L.) stocks in the Baltic Sea and neighbouring areas, and in European whitefish (*Coregonus lavaretus*) ecotypes, populations and run-timing types in Finland. Moreover, the amount and pattern of genetic diversity in historical salmon populations before human impact were described, and the proportion of diversity maintained in the present hatchery stocks evaluated.

Salmon populations in the Baltic Sea were, on average, significantly less variable than eastern Atlantic populations, and the diversity of landlocked populations (Lakes Vänern, Saimaa, Onega and Ladoga) was in turn significantly lower than that of anadromous salmon populations in the Baltic Sea populations. Within the Baltic Sea, the anadromous populations of Atlantic salmon formed three clear groups, corresponding to the northern (Gulf of Bothnia), eastern (Gulf of Finland and eastern Baltic Main Basin) and southern (western Baltic Main Basin) regions. The genetic differences among these three groups were clearly greater (G_{GB} 5.6%; G_{GB} being the proportion of diversity components between regions within basins) than those among population groups in the eastern Atlantic Ocean (G_{GB} 2.2%) from Ireland to the White Sea. The isolation-by-distance model explained part of the differentiation within, but not between, the regions. Based on microsatellite data, three salmon population groups in the Baltic Sea were considered potentially different colonization lineages and were supported by relatively high bootstrap values.

In short-term breeding programmes of Atlantic salmon, the average observed rate of loss of alleles was 4.9% per generation and the average rate of loss of heterozygosity was 1.4% per generation. The corresponding figures for long-term breeding programmes were 2.0% and <1%. When comparing the genetic parameters of stocks before and after hatchery breeding of several successive generations (Rivers Iijoki and Oulujoki), statistically significant changes in allele frequencies were common. The changes were also compared with those observed over 56 years in a large wild Atlantic salmon stock in the Teno River, a stock that remained temporally very stable. Despite the observed losses of genetic diversity in broodstock breeding, a large proportion of the genetic resources of the extirpated stocks are still conserved in the broodstocks.

Genetic differentiation among European whitefish ecotypes was generally low, thus giving support to the hypothesis of one native European whitefish species in Fennoscandia. Among the ecotypes, the northern, large sparsely rakered, bottom-dwelling whitefish was the most unique. The known genetic differences in quantitative traits have thus either developed independently of potential phylogenetic lineages, or the lineages have mixed and the quantitative traits of the ecotypes, like gill-raker number, have later changed according to environment and selection pressures. Overall, genetic distances between the anadromous whitefish populations along the Finnish coast, especially in the Bothnian Bay area, were small. Wild whitefish populations studied had slightly higher allelic diversity than hatchery-reared populations in corresponding rivers.

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Publications I-IV

Original publications

This thesis is based on the following original papers, which are referred to in the text by their Roman numerals:

- I Koljonen, M.-L., Tähtinen, J., Säisä, M., and Koskiniemi, J. 2002. Maintenance of genetic diversity of Atlantic salmon (*Salmo salar*) by captive breeding programmes and the geographic distribution of microsatellite variation. *Aquaculture* 212: 69-92.
- II Säisä, M., Rönn, J., Aho, T., Björklund, M., Pasanen, P., and Koljonen, M.-L. 2008. Genetic differentiation among European whitefish ecotypes based on microsatellite data. *Hereditas* 145: 69–83.
- III Säisä, M., Koljonen, M.-L., and Tähtinen, J. 2003. Genetic changes in Atlantic salmon stocks since historical times and the effective population size of a long-term captive breeding programme. *Conservation Genetics* 4: 613-627.
- IV Säisä, M., Koljonen, M.-L., Gross, R., Nilsson, J., Tähtinen, J., Koskiniemi, J., and Vasemägi, A. 2005. Population genetic structure and postglacial colonization of Atlantic salmon (*Salmo salar*) in the Baltic Sea area based on microsatellite DNA variation. *Canadian Journal of Fisheries and Aquatic Sciences* 62: 1887-1904.

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The author participated in the laboratory analyses of Papers I, III and IV, and in statistical analyses and writing of all papers.

1. Introduction

1.1. Atlantic salmon (*Salmo salar* L.) and European whitefish (*Coregonus lavaretus*) in the Baltic Sea area

Families Salmonidae and Coregonidae belong to the order of Salmoniformes. They are distributed throughout the Northern Hemisphere and are highly valuable in both sport and commercial fisheries. The value of the commercial catch of Atlantic salmon and whitefish from the Baltic Sea was about 1.2 and 2 million euros, respectively, and the catches totalled 309 000 and 690 000 kilos in 2006.

Atlantic salmon (*Salmo salar* L.) lives in the Western Atlantic at coast drainages from Northern Quebec, Canada, to Connecticut, USA, and in the Eastern Atlantic at drainages north of the Arctic Circle and Baltic Sea drainage to Portugal. Landlocked Atlantic salmon stocks are present in Russia, Finland, Sweden, Norway and North America. Atlantic salmon of the Baltic Sea area, called Baltic salmon, is known to be somewhat genetically distinctive. As a result of a diverse life history and strict homing behaviour from feeding migrations to their original home river, differentiated populations have evolved (e.g., McConnell *et al.* 1995, Sanchez *et al.* 1996, Koljonen *et al.* 1999, Verspoor *et al.* 1999).

Due to the morphological and ecological diversity, taxonomic classification of whitefish (*Coregonus lavaretus*) has been especially complicated. Classification of different whitefish ecotypes has usually been based on the gill-raker number of the populations, which is an inherited quantitative trait (Svårdson 1970, Hermida *et al.* 2002), and thus, less sensitive to environmental effects. When gill-raker number (varying from 18 to 56) and spawning habitat (river versus lake or sea) are used as classifying criteria for whitefish in Finland, six forms or ecotypes can be defined for practical purposes (Kallio-Nyberg and Koljonen 1988, Kaukoranta *et al.* 2000; Paper II). The forms differ clearly from each other in their geographical distribution, spawning environment, migration behaviour, growth rate, morphological traits and diet, and also in the number of gill-rakers. However, crossing between all of these forms is possible, and the resulting offspring are fertile. Current general opinion is that all native whitefishes in Fennoscandia belong to the *C. lavaretus* species (Himberg and Lehtonen 1994). The only other native Coregonid species in Finland is vendace (*Coregonus albula*). A third Coregonus species, *Coregonus peled*, has been introduced to Finland from a Russian Lake Endyr, in Siberia.

1.2. Changes in genetic structure

Differences among the Atlantic salmon and whitefish populations in Baltic Sea area have arisen by postglacial colonization and potentially by already differentiated lineages from distinct glacial refugia during the last glaciation (12 000 years before present (BP)). After postglacial colonization, drift, selection, mutation, migration and genetic bottlenecks may have further changed the genetic structure of both the Atlantic salmon and European whitefish stocks.

During the last century, human impacts have been the most powerful cause of changes for both species. Due to characteristic phases of life history in freshwater rivers and in the sea, salmon and anadromous whitefish stocks are especially vulnerable to environmental changes in both environments. In the Baltic Sea region, the potential of natural populations of both species to reproduce has been heavily affected by intensive fishing and habitat destruction. The loss of populations is mainly due to the construction of hydroelectric power plants and dams since the 1940s. As a result, only about 25 of the 90 original river stocks of Atlantic salmon remain within the Baltic Sea, and the numbers of many whitefish populations have also declined drastically. Loss of genetic diversity reduces adaptive capacity of the species and increases risk of extinction (e.g., Frankel and Soulé 1981, Frankham 1995). To compensate for the lost production and to maintain the genetic diversity of the salmon and whitefish in the Baltic Sea, several Baltic Sea countries have established captive breeding programmes with hatchery broodstocks. At the beginning of the 21st century, over 80% of all salmon smolts in the Baltic Sea were produced by hatchery rearing (Anon. 2002), either in long-term breeding programmes using long-lived hatchery broodstocks or in short-term breeding programmes using spawners from the wild.

Broodstocks are needed to produce smolts for compensation releases, to maintain the genetic material of stocks when reproduction habitats are lost, and to serve as gene banks for the remaining wild stocks as an insurance against risks such as variation in harvest escape, environmental factors and diseases. One of the main problems to be tackled by conservation programmes is the maintenance of genetic variation in broodstocks. There is always a risk that genetic diversity may be lost in hatchery rearing because of the accelerated rate of genetic drift in potentially small broodstocks (e.g., Allendorf and Phelps 1980, Cross and King 1983, Allendorf and Ryman 1987, Ryman *et al.* 1995). Changes may also occur in adaptive traits as a result of changed selective factors (Fleming and Einum 1997, Kallio-Nyberg and Koljonen 1997). Moreover, supportive releases as such may cause a decrease in genetic diversity (Ryman and Laikre 1991, Waples and Do 1994, Ryman *et al.* 1995, Tessier *et al.* 1997, Cross *et al.* 1998). These risks are well-recognized (Hillborn 1992), yet their level is seldom evaluated.

1.3. Molecular markers in genetic studies

Phenotypic traits typical for particular fish populations can be used as population identifiers; an example of such traits are the number of gill-rakers. Allozyme polymorphism, a simple and cheap method for revealing genetic variation, has provided an enormous amount of data for population genetic studies of fish (Utter 1991). Allozyme markers have also been used in studies of genetic diversity, phylogeography and genetic stock identification of Atlantic salmon and European whitefish stocks (Koljonen and Pella 1997, Koljonen *et al.* 1999, Næsje *et al.* 2004). The relatively low number of polymorphic loci and also the low number of alleles in allozyme loci (e.g., Bourke *et al.* 1997) restrict, however, the use of allozyme data for fine-scale studies of Atlantic salmon and European whitefish populations. Moreover, the neutrality of variation cannot always be assumed, as demonstrated at the mMEP-2* locus (Verspoor and Jordan 1989, Jordan and Youngson 1991).

Efficient use of direct DNA level markers became possible since polymerase chain reaction (PCR) was invented (Saiki *et al.* 1985). DNA-level markers provide tools for population genetic studies to evaluate the amount of genetic diversity and compare population structures,

phylogenetic studies, gene mapping and investigation of individual identity and relatedness. Different marker approaches provide complementary information. Among direct DNA-level information, DNA sequencing provides detailed information about the actual order of DNA bases. Mitochondrial DNA (mtDNA), mini- and microsatellites and single-nucleotide polymorphism (SNP) are commonly used markers in population genetic studies: they have also been used in genetic studies of Atlantic salmon and whitefish. Microsatellites, tandemly repeated short nucleotide motifs, provide an efficient approach for genetic research. Because they are usually located in non-coding regions, they are less subject to selection than functional markers (Queller *et al.* 1993), and thus, could serve as test data for the neutrality of allozyme information when linking to quantitative loci does not occur. The study of evolutionary history on small temporal scales may benefit from the use of more rapidly evolving genetic markers, like microsatellites, which have the potential for finer resolution of phylogenetic signals among recently diverged groups of organisms (Bowcock *et al.* 1994, Angers and Bernatchez 1998). Allelic variation of microsatellites has been used successfully to determine genetic variation in Atlantic salmon and whitefish stocks. It has been utilized to assess genetic structure of Atlantic salmon populations in different geographical areas in North America (e.g., O'Reilly *et al.* 1996, McConnell *et al.* 1997, Tessier and Bernatchez 1999) and in Europe (e.g., Sánchez *et al.* 1996, Nielsen *et al.* 1997, Nilsson 1997, Norris *et al.* 1999, Wennevik *et al.* 2004, Tonteri *et al.* 2005, Primmer *et al.* 2006., Vähä *et al.* 2007, 2008) and in both areas simultaneously (King *et al.* 2001), and to monitor temporal changes in Atlantic salmon stocks (Nielsen *et al.* 1997, Saura *et al.* 2006). In whitefishes, the extent of morphological and genetic differentiation has also been compared with the aid of microsatellites (e.g., Bernatchez *et al.* 1999, Lu and Bernatchez 1999, Lu *et al.* 2001, Østbye *et al.* 2006, Hansen *et al.* 2008).

1.4. Objectives of the study

The objectives of the study were as follows:

- To describe the extent and patterns of genetic variation in historical and present-day Atlantic salmon stocks in the Baltic Sea and neighbouring areas with the aid of microsatellite markers (Papers I, III and IV). Further, to define the relation of Atlantic salmon stocks in the Baltic Sea with other genetically differentiated groups of the species in Northern Europe and Northern America (east and west coasts of Atlantic Ocean, Barents Sea and White Sea; Papers I and IV). In addition, to assess the amount of genetic differentiation at DNA microsatellite loci in European whitefish stocks in Finland among a range of hierarchical levels and substructures (Paper II).
- To provide information on the evolutionary and postglacial colonization history of Baltic Sea basin salmon stocks. Extending the scope of salmon stock sampling outside the Baltic Sea was done to gain understanding of the global structure of Atlantic salmon stocks and their relation to the history of Atlantic salmon in the Baltic Sea (Paper IV).
- To describe the amount and pattern of genetic diversity in historical salmon populations before human impact (Paper III), and to evaluate the proportion of diversity maintained in present-day hatchery stocks of Atlantic salmon and European whitefish (Papers I-III).

2. Materials and methods

2.1. Data

The number of samples (sampled stocks) in Papers I-IV varied from 11 to 38 and the number of individuals from 665 to 2180 (Table 1).

Table 1. Basic information of Studies I-IV

Paper	Species	No. of samples	No. of individuals	Objectives
I	Atlantic salmon	12	665	To describe the extent and patterns of genetic variation in 9 microsatellite loci. To evaluate effects of broodstocks breeding. To evaluate discrimination power of microsatellite analysis compared with allozyme data.
II	Whitefish	35	1788	To describe the levels of genetic diversity and differentiation among Finnish European whitefish populations, hierarchical levels and substructures in five microsatellite loci. To evaluate effects of broodstock breeding.
III	Atlantic salmon	11	749	To assess human-caused changes in two Atlantic salmon stocks in the Baltic Sea with the aid of historical scale samples.
IV	Atlantic salmon	38	2180	To study the genetic structure of Atlantic salmon across the Baltic Sea basin and neighbouring areas. To clarify the relationships between the main reasons for the observed genetic structure.

2.1.1. Atlantic salmon

Stocks of Baltic Sea drainage and surrounding areas

Scale, adipose fin or muscular samples of anadromous Atlantic salmon populations were collected from the rivers draining into the Baltic Sea, Barents Sea, White Sea, North Sea and the East Coast of Atlantic Ocean (Figure 1; Papers I, III and IV). In addition, five samples of landlocked Atlantic salmon populations from the Baltic Sea drainage area were analysed (Paper IV). Two samples from North America, from the West Coast of Atlantic Ocean, were available to assess the relation to Europe (Baltic Sea and Northeast Atlantic), one sample from Big Brook (Michaels River, Labrador, Canada) and the other from Narraguagus River (Maine, USA).

Historical samples

Historical samples of rivers Iijoki, Oulujoki and Teno (Figure 1; Paper III) were collected from scale archives. Iijoki and Oulujoki are now closed by power plants and long-term captive breeding and releasing programmes have been established to sustain catches and to conserve the genetic resources of these two otherwise lost salmon stocks. The oldest samples from the two hatchery stocks predated the closing of the rivers and were from the original salmon stocks before major changes due to human intervention are expected to have occurred. The damming of Iijoki began in 1961 and of Oulujoki in 1941. Oulujoki was entirely closed in 1951, when the first power plant, Pyhäkoski, was completed 43 km from the mouth of the river. The present Iijoki broodstock is based on wild catches of the spawners from the river. In the stock of Oulujoki, genetic material from at least six other stocks in the Bothnian Bay area (Tornionjoki, Kemijoki and Iijoki in Finland and Indalsälven, Skellefteälven and Umeälven in Sweden) were included, to increase the diversity of the broodstock and to satisfy the need for eggs.

Teno River is the largest European river supporting Atlantic salmon stock: the average annual catch is estimated to have been 134 tonnes in 1973-1995 (Niemelä *et al.* 1999). Teno salmon served as an example of a wild population, in which the magnitude of historical changes is expected to be small. A hatchery sample of the Teno stock was also available from one year class of a newly founded broodstock; this serves as a gene bank to ensure the maintenance of genetic resources should the ectoparasite *Gyrodactylus salaris* infect the river and cause the death of the wild stock, as has happened to at least 30 Norwegian salmon stocks (Hindar *et al.* 1991). No releases of hatchery fish are currently allowed into the Teno watershed.

2.1.2. European whitefish

A total of 29 whitefish populations belonging to the species *Coregonus lavaretus* were sampled from Finnish waters, and two samples from northern Sweden, rivers Kalixälven and Råne, were collected (Figure 2; Paper II). Five of the six different whitefish ecotypes in Finland were represented in the samples (Paper II, Table 2). In addition, hatchery broodstocks of the main whitefish stocks were analysed and, if possible, compared with the respective wild populations. Moreover, one broodstock and one stocked, naturally reproducing population of Peled whitefish (*C. peled*) were also included.

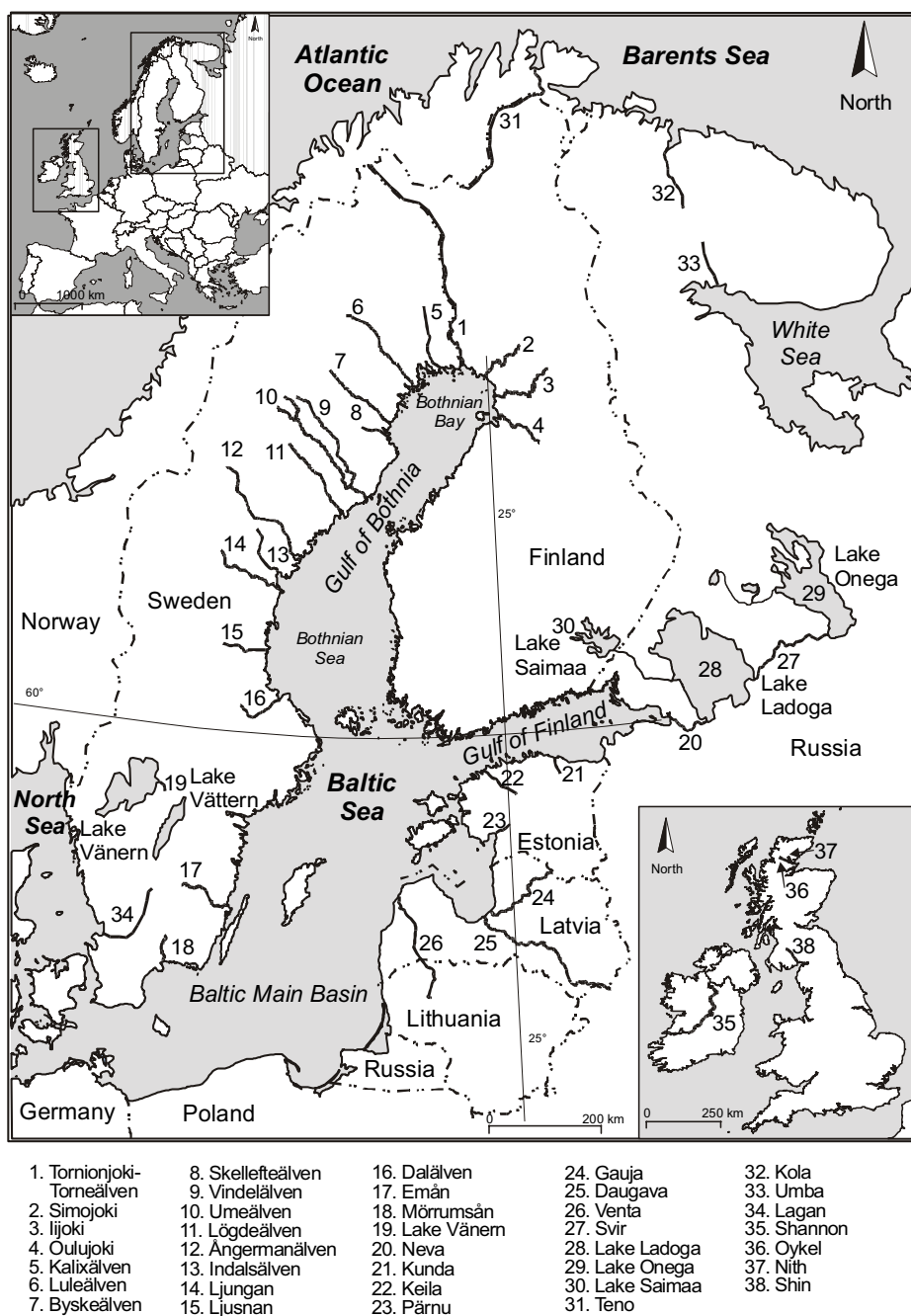


Figure 1. Studied home rivers of Atlantic salmon (*Salmo salar* L.) stocks of the Baltic Sea Basin and surrounding areas.

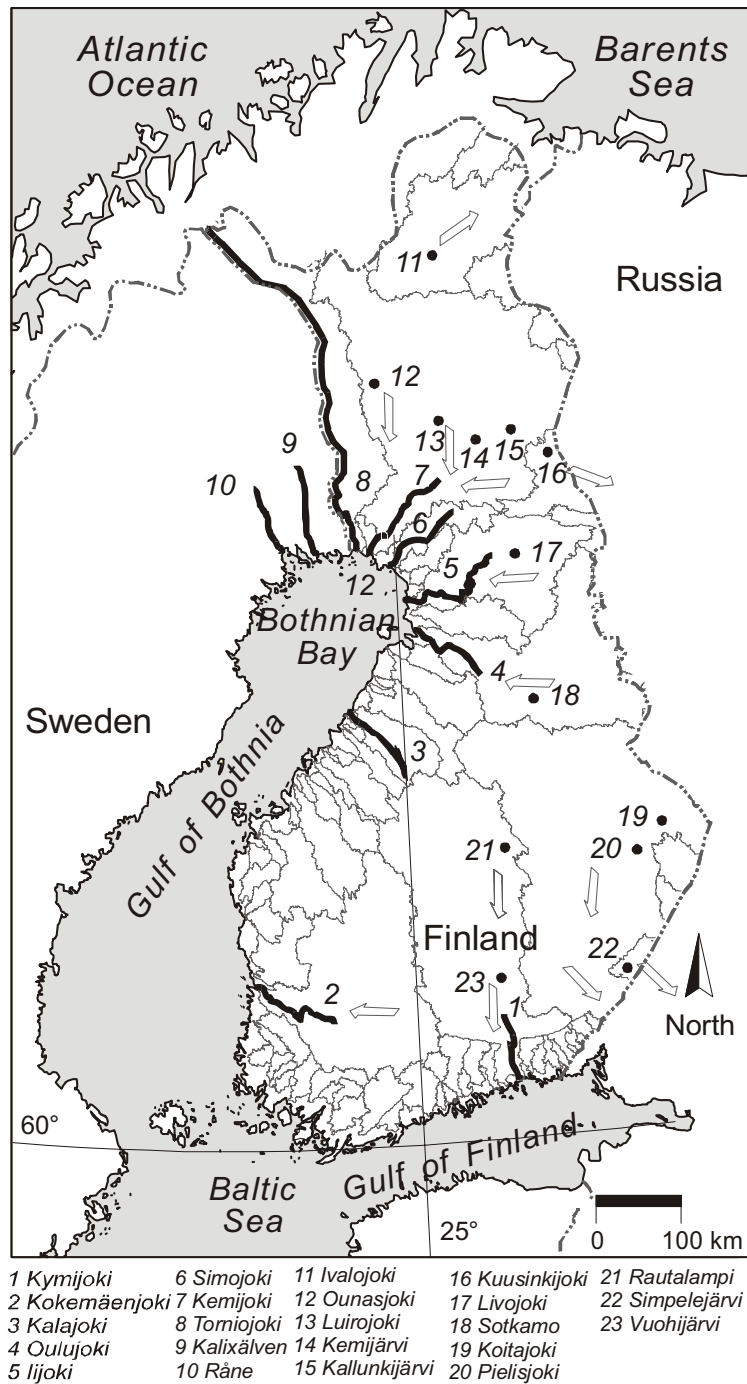


Figure 2. Origin of the Finnish and Swedish stocks of European whitefish (*Coregonus lavaretus*) studied. Boundaries of main watersheds in Finland and their flow directions are shown.

2.2. Methods

2.2.1. Laboratory analyses

Microsatellite variation in Atlantic salmon was studied at nine polymorphic loci in Papers I and IV, and at seven polymorphic loci in historical samples of Paper III. In European whitefish, five polymorphic microsatellite loci were used for the analyses (Paper II). More detailed descriptions of analyses are presented in the original papers.

2.2.2. Statistical analyses

Genetic diversity within populations

The genetic diversity for each population at each locus was measured as the number of alleles and the unbiased expected heterozygosity (H_e) (Nei 1978). Levene's (1949) correction for small sample size was used in H_e estimation. Exact tests for Hardy-Weinberg (H-W) equilibrium (Guo and Thompson 1992) were performed by the Markov chain method using the GENEPOP 3.1d and 3.2 software packages (Raymond and Rousset 1995) with Markov chain parameters 300 batches and 3000 iterations. Probabilities of Hardy-Weinberg equilibrium tests for populations were adjusted over loci using the sequential Bonferroni procedure for multiple tests (Rice 1989). Differences in mean heterozygosity between populations were evaluated by using the *t*-test for paired observations; differences in heterozygosity of individual loci were also tested according to Nei (1987). In the test for population differentiation, a total probability value over loci was obtained using Fisher's method of combining independent *p*-values.

To compare the number of alleles in samples and to obtain estimates for allelic diversity, bootstrap resampling was conducted (Paper I). The genotype distribution of each locus was resampled 1000 times with replacement for the analysed sample size. The received bootstrapped means, their standard deviations and resampling distributions were used for comparing allelic diversity in different samples. In Papers II-IV, the number of alleles in samples was compared by allelic richness (El Mousadik and Petit 1996, Petit *et al.* 1998). To compare allelic richness between samples of unequal size, adjusted estimates were calculated by rarefaction approach using FSTAT (version 2.9.3.) software (Goudet 2001). Comparisons of allelic richness between groups of samples in Paper III were analysed using permutation test (1000 permutations) with FSTAT. The significance of differences in allelic richness in Paper III was tested by the Wilcoxon signed-rank test for paired observations (Luikart *et al.* 1998). Loss of genetic diversity during hatchery breeding was evaluated as the rate of loss of mean heterozygosity and as the rate of loss of alleles.

In Papers II and IV, the populations were tested for recent reduction of effective population size (N_e) by using Wilcoxon signed-rank test as implemented in the BOTTLENECK version 1.2.02 computer program (Cornuet and Luikart 1996), assuming the two-phase model of mutation (with 5% multi-step changes and variance of 12) for microsatellite loci (Piry *et al.* 1999).

Genetic differentiation

Population differentiation was tested by the Markov chain method using the GENEPOP 3.1d and 3.2 software packages (Raymond and Rousset 1995) with Markov chain parameters 300 batches and 3000 iterations. Genetic differentiation between stocks and samples was calculated according to D_A distance based on Nei *et al.* (1983) (Papers I-IV). This method has, according to simulation studies, proved to give the most correct topology regardless of the expected mutation model (Takezaki and Nei 1996). Dendrograms were constructed by the neighbour-joining method (Saitou and Nei 1987) in Papers II-IV, and by the UPGMA method in Paper I. The neighbour-joining method does not assume a constant rate of mutations and is therefore estimated to be more appropriate than other clustering algorithms. The bootstrap test for the topology of the tree was conducted with 1000 recalculating runs using the DISPAN program package (Ota 1993). FSTAT version 2.9.3 (Goudet 2001) was used to calculate the F_{ST} values (Weir and Cockerham 1984). Standard deviation and 95% confidence limits associated with F_{ST} were assessed through bootstrapping. Hierarchical diversity analysis based on genetic diversity of European Atlantic salmon populations (Paper IV), was conducted according to Nei (1973, 1977). Isolation by distance (IBD) hypothesis of salmon stocks within Baltic Sea area in Paper IV was tested with the aid of the GENEPOP 3.2a program package (Raymond and Rousset 1995). Patterns of allele frequency variation of present-day Atlantic salmon populations were examined with multidimensional scaling (MDS) in Paper IV.

Estimates of effective population size (N_e)

Atlantic salmon is very near to semelparous as a species in the wild, as they reproduce in practice only once in a lifetime. Several year-classes contribute to the annual reproduction, and thus, the life history pattern of the species is more similar to Pacific salmonids (*Oncorhynchus spp.*) than to the brown trout (*Salmo trutta* L.); estimation of effective population size with a temporal method have been earlier applied to both (Waples and Teel 1990, Jorde and Ryman 1996). In hatcheries, however, reproduction of broodstocks occurs for several years, but generations are usually discrete, as renewal of a broodstock is not annual, and in our studies only one broodstock generation was founded and only one year-class of the offspring was analysed. The assumptions required by the model are that the generations be discrete and that selection, migration and mutation are unimportant in relation to the magnitude of drift. These assumptions were presumably met for the two salmon stocks (Iijoki and Teno) of Paper III and the two populations (Teno and Tornionjoki) of Paper I. Thus, the effective population size, N_e , was estimated with the direct model for discrete generations (Pollak 1983, Waples 1989), which in this case was assumed to be robust enough to give rough estimates of the magnitude of the effective population size (see Waples 1990). N_e was estimated in Papers I and III by the temporal method from allele frequency changes (Pollak 1983, Waples 1989, 1990a, 1990b). The maximum-likelihood based estimates of N_e in Paper II were calculated with the MLNE program (Wang 2001, Wang and Whitlock 2002).

3. Results

3.1. Population structure and genetic relationships among populations

Atlantic salmon

Allele frequencies between Atlantic salmon populations varied substantially (Paper I, Figure 2; Paper III, Figure 2; Paper IV, Figure 2). Diversity estimates based on allelic richness or mean heterozygosity were, in general, lower in the Baltic Sea salmon populations than in the Atlantic salmon populations (Paper III, Table 2; Paper IV, Table 2). Within the Baltic Sea, no significant differences in mean heterozygosity or allelic richness among populations could be observed.

All landlocked salmon populations studied (River Svir and Lakes Vänern, Saimaa, Onega and Ladoga) had lower allelic richness and mean heterozygosity estimates than anadromous populations (Paper IV). The most distinct stock was the landlocked Saimaa salmon, which is understandable considering its very small effective population size, probably fewer than 10 individuals (Kallio 1986), and the 10 000-year history of post-glacial isolation. It had the smallest number of observed alleles of all salmon populations studied, microsatellite locus *Ssa85* was even fixed for allele *134 (Paper I, Figure 2a), and it had a distinctive pattern in several other loci. In addition, the allele frequencies of landlocked population of Lake Vänern and River Neva were unique (Paper IV, Figure 2).

Microsatellite data showed a clear grouping of salmon populations. The difference between European and North American Atlantic salmon populations was quite distinct. A difference as clear as that in *SSOSL311* (single diagnostic allele with a frequency of 0.96 to 0.98) has not earlier been found in the microsatellite loci between these two continents (Paper I, Figure 2g). The genetic distance between North American (Michaels River – Big Brook, Labrador) and European salmon samples was high (average $D_A = 0.71$; Paper I, Figure 3). Within Europe, the greatest distances were observed between population pairs that included Lake Saimaa (average $D_A = 0.50$), and the shortest distances between geographically proximate Baltic Sea populations within the Eastern Main Basin ($D_A = 0.06 - 0.08$) and within the Bothnian Sea ($D_A = 0.08 - 0.11$). Atlantic salmon in the Baltic Sea differed clearly from Eastern Atlantic salmon and Barents Sea and White Sea populations (Figure 3 and multidimensional scaling analysis of D_A distance matrix; Paper IV, Figure 4). The present analysis also revealed the divergence of three main population groups of anadromous salmon within Baltic Sea; East Baltic Sea (Gulf of Finland and Eastern Main Basin), North Baltic Sea (Gulf of Bothnia) and South Baltic Sea (South-Western Main Basin) salmon. The East and North Baltic Sea groups were evident already in allozyme (Koljonen *et al.* 1999) and mtDNA (Nilsson *et al.* 2000) data. In contrast, allozyme and mtDNA data grouped South Baltic Sea salmon with East Baltic populations and with the proposed Ice Lake Lineage.

Hierarchical diversity analysis of European anadromous salmon populations revealed that only 1.9% of variation occurred between Atlantic and Baltic basins, 9.4% was due to differentiation among populations within the basins and 88.6% was due to variation within

populations (Paper IV, Table 4). Within the Baltic basin, the percentage of variation due to differences between regions was 5.6% and within regions 5.2%, leaving 89.2% of the variation to originate within populations.

Whitefish

Genetic diversity estimates for whitefish populations were high (Paper II, Table 3). *C. lavaretus* populations were clearly more polymorphic in all loci than the two *C. peled* populations. The naturally reproducing *C. peled* population studied was fixed for allele *197 in locus *BWF-1*. Among *C. lavaretus* populations, most diverse was the large population of River Kalixälven, and the lowest polymorphism was observed in the wild bottom dwelling (*C. l. fera*) population of Lake Kallunkijärvi.

In whitefish populations, the average genetic distances (D_A) between the ecotypes of *C. lavaretus* and the species *C. peled* was high, 0.86, varying between 0.82 and 0.90 among *C. lavaretus* ecotypes (Paper II, Table 4, and Figures 2 and 3). The genetic distances (D_A) among the five analysed ecotypes of *C. lavaretus* were in general clearly less than among the two species, being on average 0.15, with a range of 0.06 to 0.26 (Paper II, Table 4 and Figure 2). The average distance among ecotypes was only about 17% of that between species *C. peled* and *C. lavaretus*. However, the genetic distances between *C. l. fera* and other ecotypes were about twice as great (on average 0.22, range 0.19-0.26) as among the other four ecotypes (on average 0.10, range 0.06-0.14, for *C. l. lavaretus*, *C. l. widegreni*, *C. l. pallasi* and *C. l. nilssoni*), corresponding to 26% and 12% of the species-level differentiation.

Genetic differentiation among whitefish populations was not always strong, and in many cases no significant differences could be observed. Overall, genetic distances (D_A) among all *C. lavaretus* whitefish populations were small ($D_A=0.24$) and varied from 0.07 (wild Kemijoki autumn sample to wild Simojoki sample) to 0.55 (Rautalampi sample to Livojoki sample). The F_{ST} estimate for all *C. lavaretus* populations was 0.05, varying from 0.01 (several population pairs within the Bothnian Bay area) to 0.21 (Kallunkijärvi sample to Sotkamo sample). In a dendrogram based on D_A distances, populations from the Bothnian Bay area mainly group on the same branch, with a few exceptions (Figure 4). Populations from the same ecotype did not always cluster together.

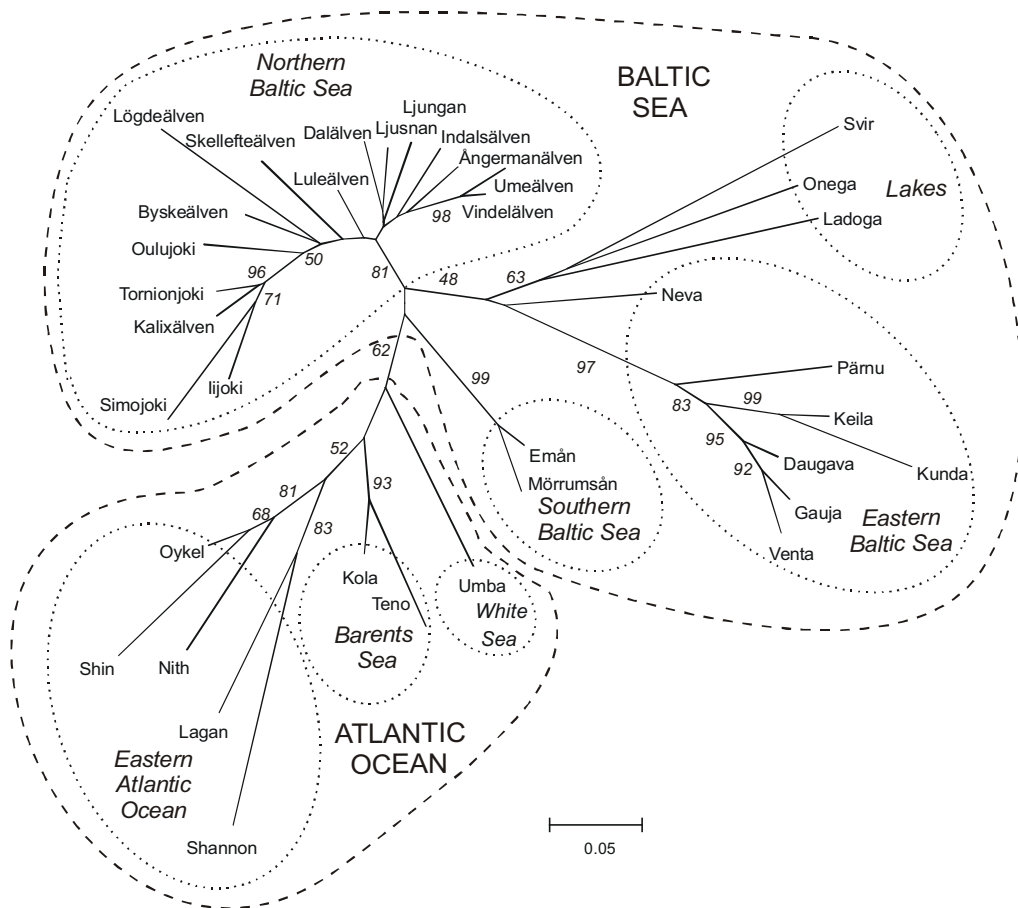


Figure 3. Unrooted neighbour-joining dendrogram of the European Atlantic salmon (*Salmo salar*) populations based on D_A distances from Nei *et al.* (1983). Major geographical groupings of populations are indicated by dotted circles. Numbers indicate branches with bootstrap support above 50% in 1000 replicates.

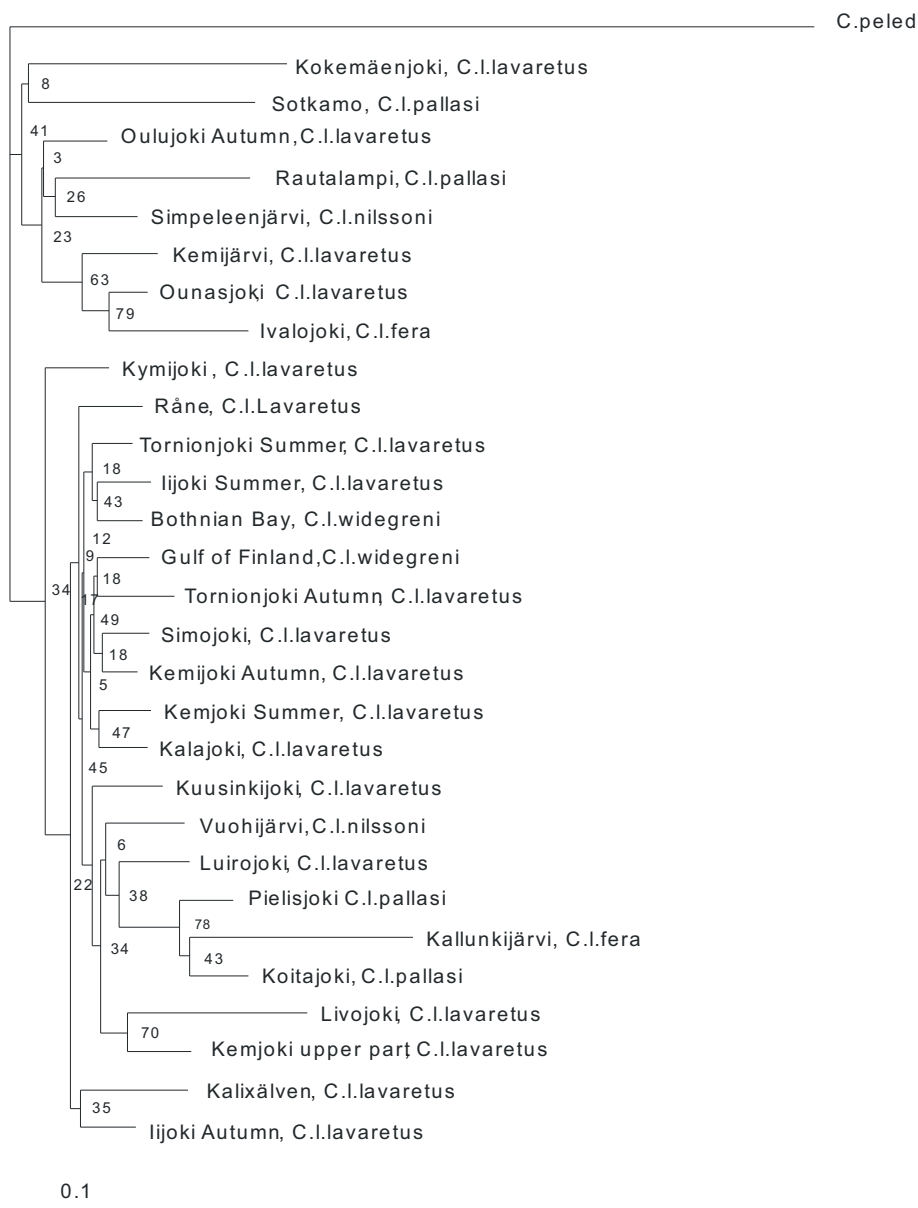


Figure 4. Neighbour-joining dendrogram (Saitou and Nei 1987) of the whitefish populations based on D_A distances. Percent support from 1000 bootstrap replications is given above each branch.

3.2. Effects of captive breeding programmes

Atlantic salmon

In short-term breeding, each broodstock generation is renewed, at least partly, with individuals from the wild population, and only second-generation hatchery offspring are released. In Paper I, samples from Teno, Tornionjoki and Simojoki hatchery stocks represented short-term breeding. Stocks of Iijoki and Oulujoki in Papers I and III represent long-term captive breeding programmes since several generations of broodstock breeding has occurred in both cases (4.5 generations in River Iijoki and 10 generations in River Oulujoki stock).

In pair-wise population differentiation tests in Papers I and III, allele frequencies in all hatchery samples differed highly significantly from the corresponding original wild populations, and in Paper III in Iijoki also between different broodstock generations. The only exception to this was the difference between the last samples of the Iijoki stock (1992-1995), a period representing only half of a generation (Paper III, Table 5). In contrast to hatchery stocks, no significant difference was observed between the oldest and more recent wild samples of the wild Teno salmon, which was temporally very stable.

Loss of allelic diversity could be observed in all broodstocks studied. The broodstock samples of salmon in Paper III had on average 9.7 (15.7%) fewer alleles than the corresponding historical samples. When comparing short- and long-term captive breeding, the average allelic diversity was somewhat higher (7%) for short-term breeding (72.8 alleles) than for long-term breeding (65.8 alleles) programmes in the Bothnian Bay area (Paper I). In the short-term broodstock breeding of the Teno and Tornionjoki stocks, the observed average rate of loss of alleles was 4.9% of the original amount (Paper I, Table 5). There was, however, a difference between the broodstocks, as the loss of alleles had occurred mainly in the Teno stock. The average loss of alleles per generation in long-term broodstock breeding of Atlantic salmon varied from 1.5% to 2.5% (Paper III, Table 3).

Comparison of wild stocks of Rivers Teno and Tornionjoki with those of their newly founded short-term broodstocks showed only a 1.7% decrease in heterozygosity (Paper I). In Bothnian Bay, the mean heterozygosity was 2%-units higher ($H_e = 70.1\%$) in the broodstocks in the short-term breeding programme (Tornionjoki and Simojoki) than in the long-term breeding programme (Iijoki and Oulujoki; $H_e = 68.1\%$) (Paper I). The observed average rate of loss of heterozygosity per generation was in short-term broodstock breeding 1.4%-units (Paper I) and in long-term broodstock breeding of 0.1 to 0.9%-units (Paper III) of the total heterozygosity, indicating that marked loss occurred in the first generation, which during continued breeding levelled down, with losses being much lower in subsequent generations.

Whitefish

Although the whitefish populations of the Bothnian Bay area were genetically very similar, the comparison of wild and hatchery-reared populations of the corresponding rivers revealed

highly significant differences in allelic frequencies (Paper II). The wild whitefish populations had on average one allele more per locus than the hatchery-reared populations (mean allelic richness 9.81 and 8.80, respectively; *C. peled* populations excluded). However, the mean heterozygosity between wild and hatchery-reared populations did not differ as much ($H_e = 0.84$ and 0.81 , respectively; *C. peled* populations excluded), the loss of heterozygosity being 1.7% (Paper II).

3.3. Estimates of effective population size

Atlantic salmon

Estimates of effective population size (N_e) based on the temporal method were calculated for Atlantic salmon broodstocks in Tornionjoki ($N_e = 238$, calculated with 9 loci; Paper I) and for different time intervals of the Iijoki broodstock (N_e varying from 13 to 79, calculated with 7 loci; Paper III). Estimates of N_e were also calculated for the vigorous wild stock of Teno (N_e exceeding 900, calculated with 7 loci; Paper III) and the corresponding broodstock ($N_e = 32$ and 44, corresponding loci used in calculations 9 and 7; Papers I and III).

Whitefish

Estimates of N_e based on the maximum likelihood method for hatchery populations of anadromous European whitefish in Rivers Oulujoki, Kymijoki and Kalajoki were less than 50 fish (33-46 fish; Paper II, Table 5). The estimate of N_e for the Iijoki hatchery population was the largest, 122 fish. The 95% confidence intervals were also greater in Iijoki than in the other two cases.

4. Discussion

4.1. Atlantic salmon

4.1.1. Population structure of Atlantic salmon

The difference between European and North American Atlantic salmon populations was clear, as expected in light of the wide geographical distance separating them and the findings of earlier studies on the topic (Reddin 1986, Ståhl 1987, Bermingham *et al.* 1991, McConnell *et al.* 1995, Taggart *et al.* 1995). Our finding of a difference as clear as that in microsatellite loci *SSOSL311* between continents (Paper I) was new. In previous microsatellite comparisons (McConnell *et al.* 1995, Norris *et al.* 1999), this locus was not studied. McConnell *et al.* (1995) had observed a difference between continents in the *Ssa289* locus. The information obtained here is useful for the analysis of stock proportions for the ocean fishery of Atlantic salmon, for example, in the waters of West Greenland to which stocks from both continents migrate to feed.

By combining the likelihood of the occurrence of alleles in several loci, it will be possible to assign with precision individual fish in catches at least to their continent of origin and possibly to their watershed of origin (Paetkau *et al.* 1995, Rannala and Mountain 1997, Beacham *et al.* 2000, Koljonen 2006). A marked differentiation was also observed among European Atlantic salmon stocks (Papers I and IV). The Atlantic salmon populations in the Baltic Sea were, on average, significantly less variable than eastern Atlantic populations, and the diversity of landlocked populations (Lakes Vänern, Saimaa, Onega and Ladoga) was in turn significantly lower than that of anadromous salmon populations in the Baltic Sea. This is in concordance with earlier studies of allozyme (Ståhl 1987) and mtDNA (Nilsson *et al.* 2001) markers and is most probably due to past population size bottlenecks and postglacial founder effects. Consuegra *et al.* (2002) noted that in European salmon the highest mtDNA nucleotide diversity was in the British Isles and proposed colonization from multiple refugia to explain this.

The large-scale genetic structure obtained for salmon (Paper IV) revealed a clear grouping of populations according to their geographical location, i.e. the eastern Atlantic (including Barents Sea and the White Sea) and the Baltic Sea basins. This is consistent with earlier findings of allozyme (Ståhl 1987, Bourke *et al.* 1997, Koljonen *et al.* 1999) and mtDNA (Verspoor *et al.* 1999, Nilsson *et al.* 2001) studies and supports the concept of effective isolation of Baltic Sea salmon populations from their Atlantic counterparts. The anadromous salmon populations within the Baltic Sea formed three distinct clusters corresponding to the northern (Gulf of Bothnia), eastern (Gulf of Finland and eastern Baltic Sea) and southern (western Main Basin) regions. The northern and eastern Baltic population clusters were also revealed by allozyme (Koljonen *et al.* 1999) and mtDNA (Nilsson *et al.* 2001) markers, but in the previous studies the southern Baltic populations (Rivers Mörrumsån and Emån) clustered with the eastern Baltic Sea populations. Differences between the three geographical groups were considerable and explained 5.6% of the total microsatellite variation or approximately half of the interpopulation variation in the Baltic Sea, magnitudes similar to those based on allozyme markers (Koljonen *et al.* 1999).

4.1.2. Postglacial colonization of salmon in the Baltic Sea

The whole Baltic Sea area was either totally or partly covered with an ice layer during the last Weichselian (Wisconsinian) glaciation (from about 100 000 to 13 000 years BP), and colonization from refugial areas was not possible until the ice cover melted. The ice sheet covered also the two largest lakes in Europe, Lakes Onega and Ladoga in Russia, although the outer margin of the glacier was less than 100 km southeast of Lake Onega (Saarnisto *et al.* 1995). Postglacial colonization dynamics of several species at Baltic Sea area has been studied with brown trout (*Salmo trutta*) (Bernatchez and Osinov 1995, García-Marín *et al.* 1999), ringed seal (*Phoca hispida*) (Forstén and Alhonen 1975, Ukkonen 1993), perch (*Perca fluviatilis*) (Nesbø *et al.* 1999), bullhead (*Cottus gobio*) (Kontula and Väinölä 2001) and grayling (*Thymallus thymallus*) (Koskinen *et al.* 2000). For the Atlantic salmon in the Baltic Sea, three hypotheses of their origin have previously been proposed: eastern refugia in preglacial lakes before the Yoldia stage (Kazakov and Titov 1991, Nilsson *et al.* 2001, Tonteri *et al.* 2005), western origin from Atlantic populations via Närke Strait at the beginning of the Yoldia stage (Verspoor *et al.* 1999) and origin from both directions (Koljonen *et al.* 1999). The hypothesis of plane western colonization of all Baltic Sea populations (Verspoor *et al.* 1999) was based on mitochondrial ND1 gene variation in European salmon populations. In that study, however, only the northern Baltic Sea populations were represented (Rivers Tornionjoki, Simojoki, Luleälven, and Dalälven); those of the eastern or southern groups were not sampled. The similarity of the northern Baltic Sea group to the Atlantic populations is in any case fully compatible with results from all markers, allozymes, mtDNA and microsatellites (Koljonen *et al.* 1999, Nilsson *et al.* 2001).

The anadromous eastern Baltic Sea group (Gulf of Finland and Eastern Main Basin) and the landlocked populations in the two Russian lakes, Onega and Ladoga, have been proposed to belong to the Ice Lake Lineage, which colonized the Baltic Sea area from an eastern refugium during the Baltic Ice Lake stage (Koljonen *et al.* 1999, Nilsson *et al.* 2001, Gross *et al.* 2003, Tonteri *et al.* 2005). Current microsatellite data fully support the close relatedness of these subgroups and also their clear divergence from the other groups. Some differentiation between the eastern lake and anadromous populations occurred and can be explained by random genetic drift. However, the possibility remains that the eastern Baltic Sea salmon originates from more than one eastern freshwater refugium.

Koljonen *et al.* (1999) proposed invasion of salmon from the Atlantic through the Närke Strait, across southern Sweden to the Northern Baltic Sea during the Yoldia stage (Figure 5). Nilsson *et al.* (2001), however, noted that one mtDNA haplotype common in the Atlantic was missing from the Baltic Sea. Moreover, the population in Lake Vänern, which once formed part of the Närke Strait, was fixed for a typical eastern Baltic haplotype. Some similarity between Lake Vänern salmon and the eastern Baltic populations was also seen in microsatellite data (Paper IV). These discrepancies might, however, be explained by the known bottleneck event. In contrast, the composition of GH-1 gene haplotypes in Lake Vänern was more similar to Atlantic than to Baltic Sea salmon populations (Gross *et al.* 2003). In all, several mitochondrial haplotypes absent from southern and eastern Baltic populations are shared with the northern Baltic Sea group and Atlantic populations (Nilsson *et al.* 2001), and the similarity of the northern Baltic Sea group and Atlantic populations is also shown by both allozyme (Koljonen *et al.* 1999) and current microsatellite data (Paper IV). Thus, entry from the Atlantic via the Närke Strait route about 11 500 years BP remains the most likely colonization hypothesis for the northern Baltic Sea group, although the original colonization lineage may later have admixed to some extent with other lineages.

The southern Baltic Sea populations of Mörrumsån and Emån formed a distinct cluster located between the Atlantic and other Baltic population clusters in the dendrogram (Figure 3), with the shortest genetic distance within the Baltic Sea being to the northern Baltic Sea group. In all, its genetic distances are shortest to the White Sea and Barents Sea populations. There is no evidence of mutational contribution to the differentiation, but because differentiation is very clear and the mtDNA haplotype composition of this southern Baltic Sea group is, in contrast to allozyme and microsatellite data, identical to that of the eastern Baltic and, further, differs considerably from both the Swedish west coast populations and the northern Baltic Sea group (Nilsson *et al.* 2001), these southern populations are here regarded as distinct lineage.

The southern Baltic Sea salmon populations were geographically closest to Atlantic waters after the Närke Strait route was closed for migration 11 000 years ago (Björck *et al.* 2002) and the Danish Straits opened. However, the Atlantic colonization direction is unlikely because the mtDNA is similar to that of the eastern populations. Moreover, neither Baltic nor Swedish west coast wild salmon pass the Danish Straits in significant numbers (Christensen and Larsson 1979). It is more likely that this area was colonized from a southern refugium during the Baltic Ice Lake stage, as it was deglaciated very early in Baltic Sea history and preglacial ice-dammed lakes are also known to have existed in the Neman, Vistula, Odra and Elbe drainage basins (Marks 2002), possibly serving as glacial refugia for salmon (Figure 5). The Atlantic salmon populations of these drainages have become extinct, and thus, no comparison with a living potential source population is possible. An older, Atlantic-type origin is further supported by marked similarity with northern Atlantic populations of the Barents Sea and White Sea areas.

The similarity in mtDNA data between certain groups (e.g., White Sea, eastern Baltic Sea group) might be attributed to their connections before the last glaciation, when they were part of the ancient Scandinavian Atlantic salmon population. Similar preglacial connections might explain the similarity of the southern and eastern groups within the Baltic Sea, even though they spent the last glaciation in different refugia. The information from mtDNA is more probably related to preglacial history.

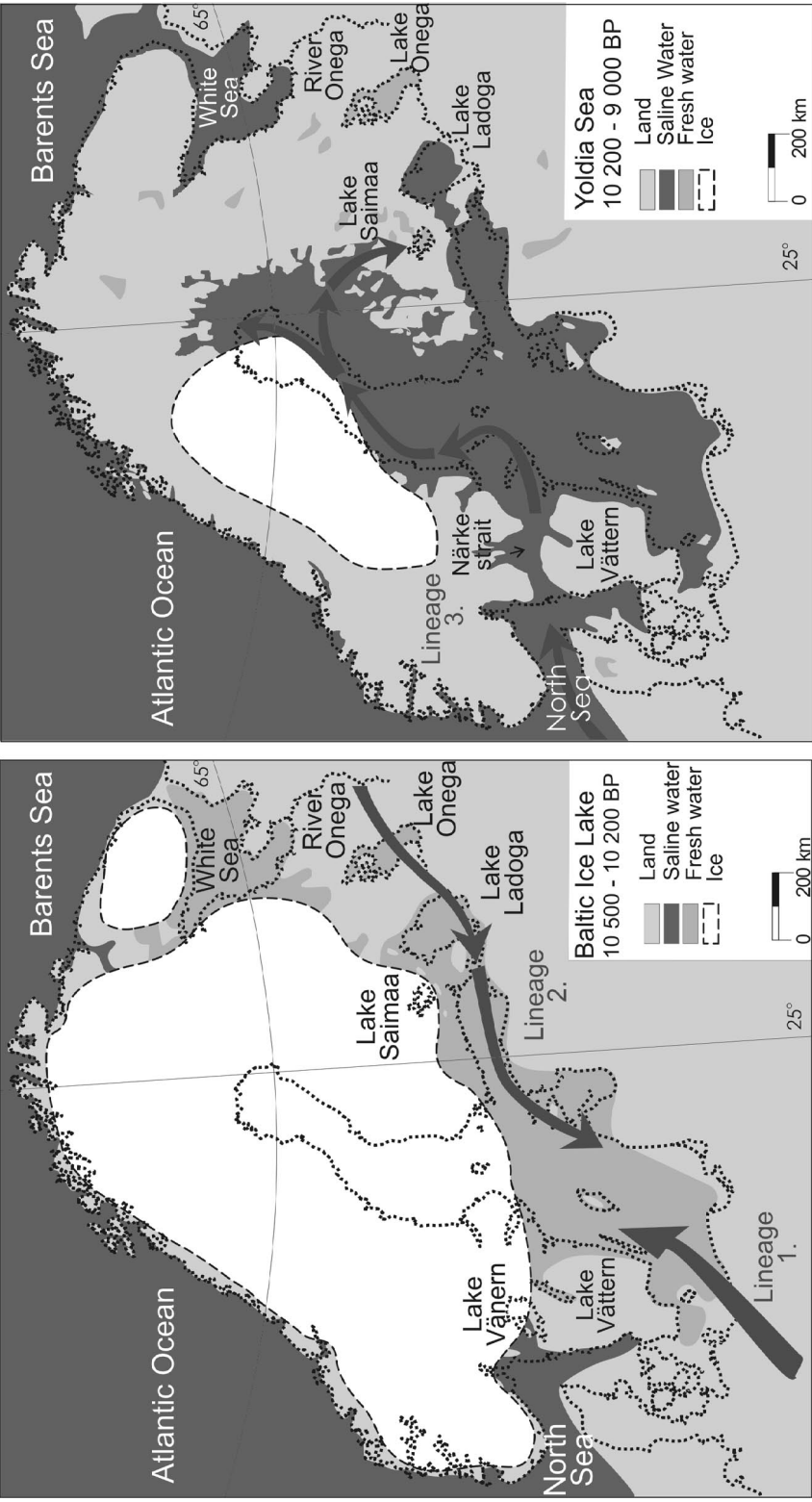


Figure 5. Phases of the last Weichselian (Wisconsinan) glaciation and three hypothetical colonization routes of Atlantic salmon to the Baltic Sea (lineages 1-3).

4.1.3. Effects of captive breeding programmes

Substantial temporal changes in allele frequencies occurred in both long-term broodstock populations of Atlantic salmon, in contrast to the wild Atlantic salmon population of Teno River, which was temporally quite stable (Paper III, Table 5). The allelic variation observed in hatchery broodstocks is, however, not necessarily entirely anthropogenic, as some population size-dependent variation is likely in smaller wild stocks as well. Allele frequencies can be expected to vary by several percentage units from year to year as a result of drift alone (Waples 1989b, Waples and Teel 1990). The original population sizes of wild Iijoki and Oulujoki salmon were probably not quite as large as that of Teno salmon, but were still at least tens of thousands, as estimated from the respective original production levels of 300 000 and 450 000 smolts. Thus, the level of genetic drift in the historical populations was probably closer to that of the contemporary wild Teno salmon than to that of present-day broodstocks. Heterozygosity has decreased significantly in the Oulujoki broodstock in 40 years, but no changes were observed in the Iijoki broodstock in 33 years. In the wild Teno salmon, both allelic richness and mean heterozygosity have remained the same for over 55 years.

A decrease of 1%-unit per generation in mean heterozygosity due to inbreeding is generally considered acceptable in livestock breeding (Franklin 1980, Frankel and Soulé 1981). The background for this criterion is that natural selection is expected to offset inbreeding depression if it is less than about 1% per generation. The observed average rate of loss of heterozygosity in short-term broodstock breeding of Atlantic salmon was 1.4%-units per generation (Paper I) and in long-term broodstock breeding below 1%-unit per generation (Paper III). Thus, according to estimates of heterozygosity in this study, no dramatic inbreeding depression effects were expected in corresponding broodstocks of Finnish anadromous Atlantic salmon. Since the studied samples were taken from routine breeding, they should represent ordinary breeding practices.

Allelic diversity is much more sensitive to population bottlenecks than is heterozygosity, as predicted by the genetic theory, especially for microsatellite loci when the allele number is high (Nei *et al.* 1975, Frankel and Soulé 1981, Leberg 1992, Luikart *et al.* 1998). Significant changes in total allele numbers were also more easily observable in this data set than were changes in mean heterozygosity. Comparison of the diversity measures of long-term broodstock breeding programmes with those of short-term programmes showed an allelic richness difference of 9.2 alleles in Atlantic salmon, but mean heterozygosities were at the same level (Paper III, Table 6).

Despite the increased resolving power of microsatellite data, the average level of heterozygosity was not significantly lower for the hatchery stocks than for the wild stocks (Papers I and III). The power of the test to observe changes at this level is, however, still relatively weak, as the number of studied loci is low in relation to the variance between loci, and only bottlenecks with very few individuals are expected to be observed (McCommas and Bryant 1990). Unlike in allozyme studies, the number of loci investigated can be increased in a microsatellite study. The probability of observing changes in mean heterozygosity is low if only a few loci are examined. With a sample size of 30, as many as 15 loci are needed to give 80% probability, i.e., power of observing a change in mean heterozygosity, caused by a bottleneck of only four pairs, in allozyme analyses (McCommas and Bryant 1990, Leberg 1992). In comparison, with the same sample size of 30, 10 microsatellite loci should provide

80% power to detect changes in allelic richness with the Wilcoxon signed-rank test, caused by a bottleneck of 10 individuals (Luikart *et al.* 1998). To increase the power to detect differences in diversity indicators, more loci should be used.

Changes in neutral genetic markers have been observed previously, too, in captive breeding (e.g., Verspoor 1988, Wilson *et al.* 1995, Norris *et al.* 1999), with some exceptions (Youngson *et al.* 1991). Neutral genetic markers provide valuable information, but they do not give information about changes in fitness traits. The correlation between molecular markers and quantitative traits has often been noticed to be weak (Karhu *et al.* 1996, Reed and Frankham 2001, McKay and Latta 2002). Hatchery breeding may also cause genetic changes in quantitative adaptive traits (Einum and Fleming 1997, Fleming and Einum 1997, Kallio-Nyberg and Koljonen 1997), such as growth rate, age at maturity, migration behaviour and domestication. Diversity measures in neutral genetic markers are useful indices, but their final evolutionary or biological significance will only become clear once their relation to fitness traits is established (Waples 1998, Hedrick 1999).

If no selection is conducted in broodstock sampling, random sampling should in principle ensure sufficient distribution in adaptive loci as well. This will not prevent the selective effects of rearing practices and the hatchery environment. The important question is, when is the observed difference in diversity level meaningful (Waples 1998, Hedrick 1999).

4.1.4. Hatchery rearing and effective population size

The success of captive breeding programmes is related to the effective population sizes (N_e s) that can be maintained in hatcheries. N_e helps to predict the loss and distribution of neutral genetic variation (Robertson 1961), and the fitness and survival of a small population (Lynch *et al.* 1995). It is therefore valuable in the management of populations of endangered species (Frankham 1995, Wang 2004). The main factors known to affect N_e are unequal sex ratio, variance in family size and fluctuations in population size over generations (e.g., Wright 1969, Falconer and Mackay 1996). Several genetic parameters, e.g., loss of heterozygosity over generations, changes in allele frequencies due to genetic drift, lethal allelism and linkage disequilibrium, can be used to estimate N_e (e.g., Nei and Tajima 1981, Pollak 1983, Waples 1989, 1990, 1991, Frankham 1995a, Falconer and Mackay 1996, Miller and Kapuscinski 1997, Wang 2005). The so-called temporal method, based on the relation of the magnitude of genetic drift to N_e , has proven promising and has been applied to salmonids (Waples 1989, Jorde and Ryman 1995, 1996). It estimates the harmonic mean N_e during the period when samples are taken. Estimation of N_e by the temporal method can be performed if the population size has been small enough to influence allelic frequencies. This method is more reliable for small populations, for which it is also more necessary (Waples 1989).

In principle, the fish studied should have constituted a representative sample of the entire generation. This could not, however, be known in sampling conducted afterwards on hatchery fish lacking pedigree information. Possibly, the N_e of each of the different hatchery populations was smaller than that of the whole generation. Pooling of populations and year-classes in later generations could potentially have increased the N_e s compared with those of sampled populations.

Although the observed N_e s of the Iijoki broodstock in Paper III are rough estimates, they indicate that in some broodstock populations, the N_e has been less than 100 fish for 4.5 generations, which is below the recommended level of 500 individuals for long-term conservation (Franklin 1980, Frankel and Soulé 1981).

4.2. Whitefish

4.2.1. Population structure of whitefish

The genetic distance between *C. peled* and *C. lavaretus* populations was 0.86 (Paper II), which supports the species-level difference between these two whitefishes. F_{ST} estimates of the ecotypes in Paper II were small, varying from 0.01 to 0.06. The genetic distances among different ecotypes were generally less than those between populations within ecotypes (Paper II, Table 4), the only exceptions being ecotype *C. l. widegreni* (sea-spawning lesser sparsely rakered whitefish) and *C. l. lavaretus* (migratory, anadromous whitefish). The most distinctive ecotype was bottom-dwelling whitefish (or northern large sparsely rakered whitefish, *C. l. fera*), with the largest genetic distance to all other groups. This ecotype also had the largest within-group distance ($D_A = 0.50$). The data support the idea that the bottom-dwelling whitefish populations generally differ more from the other four *C. lavaretus* ecotypes (*C. l. widegreni*, *C. l. lavaretus*, *C. l. nilssonii* and *C. l. pallasi*) than the other four do from each other. No separate phylogenetic background could be assumed for the four similar types on the basis of our data from neutral DNA markers (Paper II). For *C. l. fera*, the case remains open and needs more research and comprehensive sampling to be solved.

The genetic differentiation among ecotypes remained far beyond the species-level differentiation observed between *C. peled* and *C. lavaretus*, and thus, the results clearly support the existence of only one native European whitefish species in Fennoscandia. In some cases, the ecotypes may, however, reflect phylogeographic lineages within the species, especially in the case of the bottom-dwelling whitefish, *C. l. fera*, although further research is needed to verify this. Genetic differences in quantitative traits, such as gill-raker number, spawning or run timing, migration behaviour and spawning habitat preference, do exist, arising from natural selection.

Phylogenetic analysis of the European whitefish shows that the allelic frequencies differ very little, and genetic distances between the anadromous whitefish populations along the Finnish and Swedish coasts, especially in the Bothnian Bay area (Rivers Råne, Kalixälven, Tornionjoki, Kemijoki, Simojoki and Iijoki), are small (Paper II, Figure 3). Natural reproduction in these rivers has nearly ceased, and the fish populations are maintained mainly by stocking and supportive releases. These populations are also the ones that have the highest probability of mixing by natural means. Overall, genetic distances among anadromous whitefish are small, while the genetic distances among freshwater forms and between residents and anadromous populations are larger. The reason for this could be intense stocking among anadromous forms or anadromous forms more easily dispersing and hybridizing than residents. The bootstrap values for the anadromous whitefish populations (*C. l. lavaretus*) were low, and no clear conclusions can be made on their genetic structure based on the phylogenetic analysis or on the basis of D_A distances or F_{ST} values. Further studies with more loci are required to obtain more detailed estimates of this structure.

Subpopulations based on seasonal spawning-time differences can be identified within each river population of anadromous whitefish; these are known as summer and autumn whitefish. Spawning time is generally known to be a genetically determined trait (Sakamoto *et al.* 1999). In Paper II, highly significant allele distribution differences were observed between summer and autumn samples in two of the three sample pairs studied. Part of the observed microsatellite variation was therefore correlated with spawning-time differentiation, thus confirming the genetic background of spawning-time substructuring.

4.2.2. Effects of captive breeding

Although the European whitefish populations of the Bothnian Bay area in Paper II had similar allelic frequency distributions, comparison of wild and hatchery-reared populations of the corresponding rivers revealed highly significant differences in allelic frequencies. Hatchery rearing had in some cases decreased the allelic richness of the populations; wild populations had on average one allele more per locus than hatchery-reared populations (mean allelic richness 9.81 and 8.80, respectively; *C. peled* populations excluded). However, the mean heterozygosities between wild and hatchery-reared populations did not differ as notably (He 0.84 and 0.81, respectively; *C. peled* populations excluded). A bottleneck of short duration, as often is the case when founding new stocks in hatcheries, may reduce the number of alleles by eliminating rare alleles without having much effect on the level of heterozygosity. The loss of genetic variation due to small N_e could be even larger if direct selection is adopted when picking out the founder individuals. Despite the high level of maintained heterozygosity, the N_e estimates of hatchery broodstocks were somewhat low ($N_e = 33$ -122; Paper II, Table 5).

4.3. Implications of conservation

Besides the natural loss of genetic variation due to genetic drift and founder effects, human impacts have had major effects on salmon and whitefish populations in the Baltic Sea area within the last century. Genetic diversity of fish stocks is at risk in both wild and artificial environments. Preservation of genetic diversity is directly related to the intensity of fishing and its targeting of naturally reproductive stocks, and the quality and success of the massive and costly hatchery-rearing programmes. Captive breeding offers an optional tool when risks in the wild become high. Hatchery breeding is intrinsically neither good nor bad for the maintenance of genetic resources, and it can be implemented in a number of ways and with different effects on the genetic diversity of the fish stocks concerned. Essential for appraisal of its usefulness is risk analysis in both wild and hatchery environments, which is often difficult, as the risks may not be realized. In the Baltic Sea, environmental changes, such as eutrophication, and since 1992, the environmentally induced, yolk sac fry mortality syndrome of salmon, M74 (Vuorinen *et al.* 1997), pose a real risk for the future. In hatcheries, risks such as fish diseases may cause considerable damage. In conservation biology, appropriate management can be exercised only when detailed knowledge of the population is available (Wang 2005). Genetic information about differentiation and relationships among populations is a useful tool when deciding how management units should be formed (Koljonen 2001). The aims of conservation when resources are limited should be to conserve a maximum amount of genetic diversity, and to avoid gene flow and mixing between recognized hierarchical structures.

Broodstock breeding offers a viable conservation option, especially when the effective size of a wild population is decreasing below levels easily kept in hatcheries. Hatchery breeding is an important aid in maintaining endangered populations. However, wild production and evolution in the wild cannot be replaced by hatchery breeding. Genetic changes, such as adaptation to the hatchery environment or loss of adaptation to the natural environment, can potentially occur in captive populations. This may be disadvantageous to successful reintroductions (Frankham 1996). The final goal of the conservation of genetic resources should always be naturally reproductive populations. Hatchery breeding can offer no more than a temporary means of handling genetic resources.

5. Conclusions and future prospects

On the basis of this study it can be concluded:

- Microsatellites have proved to be effective markers in genetic analyses of Atlantic salmon and whitefish (Papers I-IV). In the future, the number of microsatellite loci used in analyses should be increased to improve the discrimination power and the power to detect differences in diversity indicators. This is especially the case with the anadromous European whitefish populations in the Bothnian Bay area.
- The salmon populations in the Baltic Sea were, on average, significantly less variable than eastern Atlantic populations, and the diversity of landlocked populations (Lakes Vänern, Saimaa, Onega and Ladoga) was in turn significantly lower than that of anadromous salmon populations in the Baltic Sea populations (Paper IV).
- Genetic differentiation among European whitefish ecotypes was generally low, giving support to the hypothesis of one native European whitefish species in the Nordic countries (Paper II). Among the ecotypes, the northern, large, sparsely rakered, bottom-dwelling whitefish (*C. l. fera*) was the most unique. Thus, observed genetic differences in quantitative traits have either developed independently of potential phylogenetic lineages or the lineages have mixed and the quantitative traits have later changed according to the environment and selection pressures. Overall, genetic distances between the anadromous whitefish populations along the Finnish and Swedish coasts, especially in the Bothnian Bay area, were small.
- Clear grouping of populations was observed with the Atlantic salmon populations. Based on microsatellite data, three salmon population groups in the Baltic Sea were considered potentially different colonization lineages and were supported by relatively high bootstrap values (Paper IV).
- Old scales proved to be a useful source of information in estimating temporal genetic changes of populations. The results could be used to evaluate and steer captive breeding programmes (Paper III).
- Despite the observed losses of genetic diversity in broodstock breeding, a large proportion of the genetic resources of the extirpated Atlantic salmon and whitefish stocks was conserved in broodstocks (Papers I-III).
- Further research is needed to reveal the wider-scale genetic structure of European whitefish stocks.
- Information produced in this study could be used in conservation and management of fish stocks of both species.

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Printing errors in papers II and III

Paper	Page	Chapter	Erroneous text	Correction
II	74		10 ml and 1 ml	10 μ l and 1 μ l
III	618	Allelic diversity	...1.2 alleles at nine loci...	...1.2 alleles at seven loci...
III	Table 3		...at nine microsatellite loci...	...at seven...